(*a*) *Buffer.* In lieu of the buffer described in § 442.40(b)(1)(ii) (*b*)(*2*) of this chapter, use the buffer prepared as follows: Dissolve 200 grams of primary standard tris (hydroxymethyl) aminomethane in sufficient distilled water to make 1 liter. Filter before use.

(*b*) *Preparation of working standard solution.* Use the ampicillin working standard. Dissolve and dilute an accurately weighed portion of the ampicillin working standard in sufficient distilled water to obtain a concentration of 1.25 milligrams of ampicillin per milliliter.

(*c*) *Preparation of sample solution.* Dissolve and dilute an accurately weighed portion of the sample with sufficient distilled water to obtain a concentration of 1.25 milligrams of ampicillin per milliliter (estimated).

(*d*) *Calculations.* Calculate the ampicillin content in micrograms per milligram as follows:

$$\text{Ampicillin content in micrograms per milligram} = \frac{A_u \times P_a}{A_s \times W_u}$$

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except use the ampicillin working standard.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 20 milligrams per milliliter.

(5) *Identity.* Proceed as directed in § 436.330 of this chapter.

[46 FR 25603, May 8, 1981, as amended at 50 FR 19918, May 13, 1985]

§ 440.9a Sterile ampicillin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile ampicillin sodium is the sodium salt of D(-)$_\alpha$-aminobenzyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 845 micrograms and not more than 988 micrograms of ampicillin per milligram on an anhydrous basis. If it is packaged for dispensing, it contains not less than 90 percent and not more than 115 percent of the number of milligrams of ampicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 2 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 8.0 and not more than 10.0.

(vii) Its ampicillin content is not less than 84.5 percent, except if the high-performance liquid chromatographic (HPLC) assay method is used, then the ampicillin content standard is not applicable.

(viii) The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than 6, except if the HPLC assay method is used, then the concordance standard is not applicable.

(ix) It is crystalline.

(x) It passes the identity test for ampicillin sodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in manufacturing another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers or if each vial contains 250 milligrams or less of ampicillin a minimum of 24 vials.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter (estimated), for the microbiological agar diffusion assay and in 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), for the iodometric assay or for the hydroxylamine colorimetric assay to give a stock solution of convenient concentration. For the high-performance liquid chromatographic assay (HPLC), transfer an accurately weighed portion of ampicillin, equivalent to about 100 milligrams of anhydrous ampicillin, to a 100-milliliter volumetric flask. Add about 75 milliliters of diluent (prepared as described in paragraph (b)(1)(ii)(*d*)(*1*)(*ii*) of this section), shake and sonicate, if necessary, to achieve complete dissolution. Also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either sterile distilled water, solution 1, or HPLC diluent to give a stock solution as specified above.

(ii) *Assay procedure.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(*d*) *HPLC assay.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a 4-millimeter X 5-centimeter guard column containing 40- to 60-micrometer diameter packing material as described for the analytical column, a 4-millimeter X 30-centimeter analytical column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silica, and a flow rate of about 2.0 milliliters per minute. Separately inject equal volumes (about 20 microliters) of the working standard preparation and the sample solution into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Reagents, working standard and resolution test solution, system suitability requirements, and calculations are as follows:

(*1*) *Reagents*—(*i*) *Mobile phase.* Prepare a suitably filtered and degassed mixture of water, acetonitrile, 1.0*M* monobasic potassium phosphate, and 1.0*N* acetic acid (909:80:10:1).

(*ii*) *Diluent.* Mix 10 milliliters of 1.0*M* monobasic potassium and 1 milliliter of 1.0*N* acetic acid, dilute with water to make 1,000 milliliters, and mix.

(*2*) *Preparation of working and internal standard solutions*—(*i*) *Working standard solution.* Dissolve a portion of ampicillin working standard, accurately weighed, in the diluent to obtain a solution having a known concentration of about 1 milligram per milliliter. Shake and sonicate, if necessary, to achieve complete dissolution. Use this solution promptly after preparation.

(*ii*) *Resolution test solution.* Dissolve caffeine in working standard solution to obtain a solution containing about 1 milligram per milliliter.

(*3*) *System suitability requirements*—(*i*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.4 at 5 percent of peak height.

(*ii*) *Resolution.* The resolution (*R*) between the caffeine and the ampicillin peaks is satisfactory if it is not less

than 2.0. The relative retention times are about 2.0 for caffeine and 1.0 for ampicillin.

(*iii*) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(i) of this section should not be changed.

(*4*) *Calculations.* Calculate the micrograms of ampicillin per milligram of sample as follows:

$$\text{Micrograms of ampicillin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the ampicillin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ampicillin peak in the chromatogram of the ampicillin working standard;

P_s=Ampicillin activity in the ampicillin working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20 milligrams of ampicillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of ampicillin per milliliter.

(7) *Ampicillin content.* Proceed as directed in § 436.213 of this chapter, using the titration procedure described in paragraph (e)(2) of that section. Calculate the ampicillin content as follow

$$\text{Percent ampicillin content} = \frac{(\text{A} - \text{B})(\text{normality of perchloric acid reagent})(174.7)(100)(100)}{(\text{Weight of sample in milligrams})(100 - m)}$$

where:

A=Milliliters of perchloric acid reagent used in titrating the sample;

B=Milliliters of perchloric acid reagent used in titrating the blank;

m=Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}$$

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using the method described in paragraph (b)(2) of that section.

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